VHZ FOR DIAGNOSIS AND TREATMENT OF CANCERS

FIELD

The present invention relates to the fields of medicine, cell biology, molecular biology and genetics. This invention relates to the field of medicine.

In particular, it relates to treatment and diagnosis of diseases such as colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, as well as compositions for such use.

BACKGROUND

VHZ is a phosphatase that shares about 28% amino acid sequence identity with human PRL-PTPs. VHZ was previously reported to be expressed in many tissues and located in the cytosol and in nucleoli (Alonso et al., 2004a).

However, the role of VHZ was largely unknown; despite its conservation through evolution with orthologues in frogs, fish, fly, and the Archaea. VHZ, as well as VHR, belongs to a separate subgroup of VH1-like PTPs (Alonso et al, 2004b). VHR has been reported to have a function in regulating cell cycle progression (Rahmouni et al., 2006).

Cancer is a serious health problem across the world. It is estimated that 7.6 million people in the world died of cancer in 2007. In the UK for example, cancer is responsible for 126,000 deaths per year. One in four people die from cancer. Known treatments for cancer include surgery, chemotherapy and radiotherapy. Many cancers can be cured if detected early enough.

There is a need for improved cancer detection and therapy.

SUMMARY

According to a 1st aspect of the present invention, we provide the use of an anti-VHZ antibody in the preparation of a pharmaceutical composition for the treatment, prophylaxis or alleviation of an invasive or metastatic cancer selected from the

group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer.

Further disclosed is VHZ for use in a method of treatment, prophylaxis or alleviation of a cancer in an individual selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva,

cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer.

There is provided, according to an example~~a 2nd aspect of the present invention~~, an anti-VHZ agent for the treatment, prophylaxis or alleviation of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, preferably in which the anti-VHZ agent is capable of down-regulating any combination of the expression, amount or activity of a VHZ sequence shown as GenBank accession number NM_017823 or NP_060293, or a sequence which has at least 90% sequence identity to that sequence, the anti-VHZ agent preferably comprising an anti-VHZ antibody such as an anti-peptide antibody generated against RRLRPGSIETYEQEK corresponding to amino acid residues (126-140) of human VHZ, such as chicken anti-human VHZ antibody (catalogue numbers LS-C32281, amino acids 35 to 90, LS-C42458, LS-A6806 and LS-A6803, LS-C32281, LifeSpan Inc, Seattle, Washington, USA), rabbit anti-human VHZ antibody (catalogue number DS-PB-00676, RayBiotech Inc, Norcross, Georgia, USA), chicken anti-human VHZ antibody (catalogue number XW-7857, ProSci Incorporated, Poway, California, USA), rabbit anti-human VHZ antibody (catalogue number F4560 and D9840-66A, United States Biological, Swampscott, Massachusetts, USA), chicken anti-human VHZ antibody (catalogue number D9840-66, United States Biological, Swampscott, Massachusetts, USA), rabbit anti-human VHZ antibody (catalogue number AHP1142, AdB Serotec, Oxford, United Kingdom), rabbit anti-human VHZ antibody (catalogue number NB110-40452, Novus Biologicals, Littleton, Colorado, USA), chicken anti-human VHZ antibody (catalogue number NB100-75328, Novus Biologicals, Littleton, Colorado, USA), or is capable of downregulating VHZ by RNA interference, such as comprising a Small Interfering RNA (siRNA), Short Hairpin RNA (shRNA), or Chimera RNAi such as a DUSP23 Pre-design Chimera RNAi (catalogue number H00054935-R01, Novus Biologicals, Littleton, Colorado, USA).

We disclose~~provide~~, in one example~~according to a 3rd aspect of the present invention~~, a kit for detecting a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary

cancer, prostate cancer and testicular cancer in an individual or susceptibility of the individual to such a cancer comprising means for detection of VHZ expression in the individual or a sample taken from him or her, preferably in which the means for detection is selected from the group consisting of: a VHZ polynucleotide or a fragment thereof; a complementary nucleotide sequence to VHZ nucleic acid or a fragment thereof; a VHZ polypeptide or a fragment thereof , or anti-VHZ antibody or an anti-VHZ agent as disclosed herein~~according to Claim 2~~, and optionally instructions for use, preferably further comprising a therapeutic drug for treatment, prophylaxis or alleviation of the cancer.

As a 2nd~~4th~~ aspect of the present invention, there is provided a method selected from the group consisting of:

(a) a method of detecting a metastatic cancer cell selected from the group consisting of: a colon cancer cell, a lung cancer cell, a squamous cell carcinoma cell including lip, larynx, vulva, cervix and penis cancer, a pancreatic cancer cell, a brain cancer cell, an oesophageal cancer cell, a stomach cancer cell, a bladder cancer cell, a kidney cancer cell, a skin cancer cell, an ovary cancer cell, a prostate cancer cell and a testicular cancer cell, the method comprising detecting, in a cell or tissue sample obtained from an individual, up-regulation of expression and/or amount and/or activity of VHZ in the cell, preferably in which the expression of VHZ is compared to the expression and/or amount and/or activity of VHZ in a control cell known to be non-cancerous;

(b) a method of determining the proliferative state of a metastatic cancer cell selected from the group consisting of: a colon cancer cell, a lung cancer cell, a squamous cell carcinoma cell including lip, larynx, vulva, cervix and penis cancer, a pancreatic cancer cell, a brain cancer cell, an oesophageal cancer cell, a stomach cancer cell, a bladder cancer cell, a kidney cancer cell, a skin cancer cell, an ovary cancer cell, a prostate cancer cell and a testicular cancer cell, or determining the likelihood that the cancer cell will become invasive or aggressive, the method comprising detecting, in a cell or tissue sample obtained from an individual, up-regulation of expression, amount or activity of VHZ in the cell;

(c) a method of predicting a survival rate of an individual with a metastatic cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising detecting, in a cell or tissue sample obtained from the individual, up-regulation of expression of VHZ in a cell of the individual; and

(d) a method of choosing a therapy for an individual with a metastatic cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising detecting, in a cell or tissue sample obtained from the individual, up-regulation of expression of VHZ in a cell of the individual and choosing an appropriate therapy based on the aggressiveness of the metastatic cancer.

We further disclose (a) a method of detecting a cancer cell selected from the group consisting of: a colon cancer cell, a lung cancer cell, a squamous cell carcinoma cell including lip, larynx, vulva, cervix and penis cancer, a pancreatic cancer cell, a brain cancer cell, an oesophageal cancer cell, a stomach cancer cell, a bladder cancer cell, a kidney cancer cell, a skin cancer cell, an ovary cancer cell, a prostate cancer cell and a testicular cancer cell, the method comprising detecting modulation (preferably up-regulation) of expression, amount or activity of VHZ in the cell, preferably in which the expression of VHZ is compared to the expression, amount or activity of VHZ in a control cell known to be non-cancerous; (b) a method according to (a) above, in which the method comprises detecting a VHZ nucleic acid, such as by means of a probe comprising at least a portion of a nucleic acid having a sequence shown as GenBank accession number NM_017823 or NP_060293 or a sequence having at least 90% sequence identity to such a sequence, or in which the method comprises detecting a VHZ polypeptide, such as by means of an anti-VHZ antibody as disclosed hereinset out in Claim 2, and preferably comprising histological grading, such as by using the Elston-Ellis modified Scarff, Bloom, Richardson grading system (Nottingham Grading System (NGS)); (c) a method of

determining the proliferative state of a cancer cell selected from the group consisting of: a colon cancer cell, a lung cancer cell, a squamous cell carcinoma cell including lip, larynx, vulva, cervix and penis cancer, a pancreatic cancer cell, a brain cancer cell, an oesophageal cancer cell, a stomach cancer cell, a bladder cancer cell, a kidney cancer cell, a skin cancer cell, an ovary cancer cell, a prostate cancer cell and a testicular cancer cell, or determining the likelihood that the cancer cell will become invasive or aggressive, the method comprising detecting modulation of expression, amount or activity of VHZ in the cell; (d) a method of

predicting a survival rate of an individual with a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising detecting modulation of expression of VHZ in a cell of the individual; (e) a method of choosing a therapy for an individual with a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising detecting modulation of expression of VHZ in a cell of the individual choosing an appropriate therapy, such as an anti-VHZ agent, based on the aggressiveness of the cancer; (f) a method of determining the likelihood of success of a particular therapy in an individual with a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising comparing the therapy with a therapy determined by a method according to (e) above; and (g) a method according to any of (a) to (f) above, further comprising a feature as disclosed herein~~set out in any of the 1st, 2nd or 3rd aspect of the invention~~.

We provide, according to an example~~a 5th aspect of the present invention~~, a method of manipulating a colon cancer cell, a lung cancer cell, a squamous cell carcinoma cell including lip, larynx, vulva, cervix and penis cancer, a pancreatic cancer cell, a brain cancer cell, an oesophageal cancer cell, a stomach cancer cell, a bladder cancer cell, a kidney cancer cell, a skin cancer cell, an ovary cancer cell, a prostate cancer cell and a testicular cancer cell: (a) the method comprising modulating (preferably down-regulating) the expression, amount or activity of VHZ in the cell, for example by exposing the cell to an siRNA, shRNA or Chimera RNAi capable of specifically binding to VHZ, or by exposing the cell to an anti-VHZ antibody as disclosed herein~~set out in Claim 2~~, preferably such that the cancer cell becomes non-cancerous or the invasive or metastatic cancer cell becomes non-invasive or non-metastatic as a result of the manipulation; or (b) the method comprising the steps of: (a) detecting increased VHZ expression, amount or activity in a cell; and (b) reducing the level of VHZ in the cell.

The present invention, in a 3rd ~~6th~~ aspect, there is provided a method of identifying a molecule suitable for the treatment, prophylaxis or alleviation of a metastatic cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising determining if a candidate molecule is an antagonist of VHZ or a sequence having at least 90% sequence identity thereto, the method involving exposing a candidate molecule to a VHZ polypeptide or a cell expressing a VHZ polypeptide in order to determine if the candidate molecule is an antagonist thereof. We further disclose ~~provides a method selected from the group consisting of:~~ (a) a method of identifying a molecule capable of binding to a VHZ polypeptide, the method comprising contacting a VHZ polypeptide or a sequence having at least 90% sequence identity thereto with a candidate molecule and determining whether the candidate molecule binds to the VHZ polypeptide or sequence having at least 90% sequence identity thereto; (b) a method of identifying a modulator of VHZ, the method comprising contacting a cell with a candidate molecule and detecting elevated or reduced expression, amount or activity of VHZ in or of the cell; (c) a method of identifying a molecule suitable for the treatment, prophylaxis or alleviation of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer, the method comprising determining if a candidate molecule is an agonist or antagonist of VHZ or a sequence having at least 90% sequence identity thereto, preferably by exposing a candidate molecule to a VHZ polypeptide or a cell expressing a VHZ polypeptide in order to determine if the candidate molecule is an agonist or antagonist thereof; and (d) a method of identifying an agonist or antagonist of a VHZ or a sequence having at least 90% sequence identity thereto, the method comprising administering a candidate molecule to an animal and determining whether the animal exhibits increased or decreased expression, amount or activity of VHZ.

We disclose, in one example, ~~In a 7th aspect of the present invention, there is provided~~ a molecule, agonist or antagonist of a VHZ polypeptide identified by a method or use as set out above.

According to an example~~8th aspect of the present invention~~, we provide a molecule capable of modulating, such as down-regulating, the expression of a VHZ for use in the treatment, prophylaxis or alleviation of cancer, such as an anti-peptide antibody generated against RRLRPGSIETYEQEK corresponding to amino acid residues (126-140) of human VHZ.

We provide an example of~~, according to a 9th aspect of the invention,~~ an antibody capable of binding to an VHZ polypeptide, in which the antibody is capable of binding to an epitope bound by antibody 209, or a variant, homologue, derivative or fragment thereof, in which the antibody preferably: (a) is capable of binding to an epitope on a VHZ polypeptide bound by antibody 209; (b) comprises an anti-VHZ antibody capable of binding to an epitope being a sequence

comprising residue C95 of VHZ, or a variant, homologue, derivative or fragment thereof; (c) comprises the variable region of monoclonal antibody 209, or a variant, homologue, derivative or fragment thereof which is capable of binding VHZ; (d) is capable of binding to an intracellular VHZ polypeptide; (e) is capable of crossing the plasma membrane of a cell; (f) is capable of binding to and inhibiting a biological activity of VHZ; (g) is capable of preventing metastasis of a cancer, such as colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer; or (h) comprises a monoclonal antibody or a humanised monoclonal antibody.

There is provided an example of~~, in accordance with a 10th aspect of the present invention,~~ a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5, or a variant, homologue, derivative or fragment thereof which is capable of binding VHZ.

As disclosed in one example~~an 11th aspect of the invention~~, we provide a nucleic acid comprising a sequence capable of encoding a molecule as set out above or a variant, homologue, derivative or fragment thereof which is capable of encoding a polypeptide having VHZ binding activity.

We provide, according to an example~~a 12th aspect of the invention~~, there is provided a cell comprising or transformed with a nucleic acid sequence as set out above or a descendent of such a cell.

According to a 4th~~13th~~ aspect of the present invention, there is provided a method of determining whether a tumour of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer in an individual is, or is likely to be, an invasive or metastatic tumour, the method comprising detecting, in a cell or tissue sample obtained from the individual, up-regulation of expression, amount or activity of VHZ in a tumour cell of the individual. Further disclosed is ~~we provide~~ a method of

determining whether a tumour of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer in an individual is, or is likely to be, an invasive or metastatic tumour, the method comprising detecting modulation of expression, amount or activity of VHZ in a tumour cell of the individual.

We disclose, in one example~~There is provided, according to a 14th aspect of the present invention~~, a method of treatment, prophylaxis or alleviation or diagnosis of a cancer selected from the group

consisting of: (a) a method of treatment, prophylaxis or alleviation of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer in an individual, the method comprising modulating the expression, amount or activity of a VHZ in a cell of an individual, preferably in which the expression, amount or activity of VHZ is decreased in a cancer cell of the individual; and (b) a method of diagnosis of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer or susceptibility to such a cancer in an individual or prognosis of an individual with such a cancer, the method comprising detecting modulation of expression, amount or activity of VHZ in a cell of the individual.

We disclose, in one example~~provide, according to a 15th aspect of the present invention~~, a method of treatment, prophylaxis or alleviation of a cancer selected from the group consisting of: colon cancer, lung cancer, squamous cell carcinoma including lip, larynx, vulva, cervix and penis cancer, pancreatic cancer, brain cancer, oesophageal cancer, stomach cancer, bladder cancer, kidney cancer, skin cancer, ovary cancer, prostate cancer and testicular cancer in an individual, the method comprising detecting modulation of expression, amount or activity of VHZ in a cell of the individual and administering an appropriate therapy, such as an anti-VHZ agent, to the individual based on the aggressiveness of the tumour.

The diagnosis, prognosis or choice of therapy may be further determined by assessing the size of the tumour, or the lymph node stage, or both, optionally together or in combination with other risk factors, preferably in which the diagnosis, prognosis or choice of therapy is further determined by assessing the oestrogen receptor (ER) status of the tumour.

The practice of the present disclosure~~invention~~ will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3,